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Short Communication

Study of pollen tube growth, cross-compatibility and fruit set in some almond genotypes

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Almond is one of the most important nut crops of Maragheh, Iran. In the present study, six Maragheh indigenous genotypes with favorable traits were selected for the investigations of pollen tube growth, fruit set and their compatibility. Genotypes were pollinated by the pollen of each other. Fruit set was studied in the filed, and pollen tube growth was studied in the laboratory with florescence microscopy. Experimental design was completely randomized with different number of treatments (pollen type) and 4 repeats in all of the crosses. Data were analyzed with SAS software. Results showed that, all of the genotypes were cross compatible, although the pollen tube number in the ovary was affected by the pollen type but fruit set was not affected.

Key words: Almond, cross-compatibility, pollen tube growth, florescence microscopy, fruit set.

INTRODUCTION

Pollination and fertilization with suitable pollinizers are efficient factors of fruit set in self-incompatible plants such as almond. High yields in almond need to plant at least two cross-compatible cultivars with overlapping blooming time and favorable effects on each other fruits (Oukabli et al., 2000 and 2002).

Traditional filed and laboratory controlled pollination, florescence microscopy studies and evaluation of pollen tube growth, beside molecular methods have been used in order to identify the self- and cross-(in)compatibility of cultivars/genotypes (Alonos and Socias i Company. 2005, Socias i Company and Felipe, 1987; Kester et al., 1994), to obtained the effective pollination period (EPP) (Ortega and Dicenta., 2002, 2004, 2006), and for studying the effects of pollen types on the fruit set, fruit quality and seed quality in almond (Kodad and Socias i Company 2008; Oukabli et al., 2000, 2002; Socias i Company and Felipe, 1987).

Socias i Company and Felipe (1987) studied the effects of pollen type on fruit set in 'Tuono' self-compatible almond cultivar and resulted that, 'Tuono' had higher fruit set following cross-pollination than self pollination. Dicenta et al. (2002) studied several fruit characteristics after self- and cross-pollination in several self-compatible almond cultivars and showed no differences between both pollination types for any of the studied fruit traits.

The objectives of this research were to study the pollen type effects on fruit set by means of the described methods in six Maragheh indigenous almond genotypes and identify their compatibility relationships.

MATERIALS AND METHODS

This study was carried out using 6 self-incompatible and late bloom indigenous genotypes of Maragheh. Genotypes were pollinated by the pollens of each other.

Pollens were collected from the flower buds with drying the pollen sacs separated from the stamens in the D felekinger stage of blooming time and stored in the 4ºC in refrigerator until it were use later.

Pollen germination was carried out in an *in vitro* medium in the Petri-dish with 1.5% agar and 15% sucrose in the dark condition inside the growth chamber at 22ºC and after 24 h of their growth protected with chloroform. Seven microscopic areas were counted randomly for evaluation of germinated pollen percentage and length of pollen tubes using a light microscope. In spring 2009, for each cross 4 are repeated and regarded, and in each repeat at least 2 branches with 60 -100 flower buds at 'D' (pre- blooming of flower buds) stage were bagged. 15 crosses were programmed as follow 100 × 101, 100 × 102, 100 × 103, 100 × 104, 100 × 105, 101 × 102,

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Abbreviations: EPP, Effective pollination period; **FAA,** formaldehyde acetic acid and alcohol; **CRD,** completely randomized design.

Table 1. Analysis of variances of the pollen germination percentage (PGP) and pollen tube length (PTL) in six studied genotypes tested in the *in vitro* medium.

SOV	DF	PGP %	PTL (µm)
Genotypes	5	1234.9**	120035.6**
Experimental error	20	43	3756
CV		14	16.1

Table 2. Comparison of means of the pollen grain germination percentage (PGP) and pollen tube length (PTL) in six studied genotypes tested in the *in vitro* medium.

Genotype	PGP %	PTL (µm)
100	45.3^{d}	749. ^{b}
101	61.2^{c}	566.2^{bc}
102	35 ^{c}	637.8^{c}
103	82^{a}	817.8^{a}
104	39.5^{e}	503.2^{bc}
105	68^{b}	640.2^{c}

(Means in each column with same letters are not significantly different at 5% level).

101 × 103, 101 × 104, 101 × 105, 102 × 103, 102 × 104, 102 × 105, 103 × 104, 103 × 105 and 103 × 105.

Flowers were pollinated when the pistils were acceptable for pollens. Branches on each tree were labeled and the percentages of initial and final fruit set were noted 4 and 8 weeks after pollination, respectively. After 5 - 6 days from pollination, pistils were collected and fixed in (FAA (formaldehyde 40%, acetic acid and alcohol 70%) solution and prepared for fluorescence microscopy observation as indicated in Ortega and Dicenta (2006).

Experimental design was completely randomized (CRD), different treatment (crosses) in 4 are repeated. The data were analyzed using SAS software (Version 9.1). Mean values were analyzed by Duncan's multiple range.

RESULTS

Pollen tube growth and germination

Analysis of variance indicated the significant differences for percentage of pollen germination and pollen tube growth in the 6 studied genotypes. Means of pollen germination percentage were ranged among 35 - 82% in the *in vitro* medium and means of pollen tube length was ranged among 503.2 - 817.8 µm, respectively, (Table 1 and 2).

Means of germinated pollen–percentage in the stigma of cross-pollinated pistils was 51.9 -89.5%. All of the crosses in each group showed significant differences in pollen grain germination percentage in the stigma, pollen tube number in the first, second and third section of the style and pollen tube number in the ovary (data not shown). The number of pollen tubes in the ovary had significant differences in the crosses (Table 3). However, pollen germination in the stigma had no correlation with pollen tube number reaching the ovary.

Fruit set

Analysis of variances and comparison of the means were carried out in crosses separately. Results showed that initial fruit set means were 40 - 67.52% in the crosses as well; mean of fruit drop percentage was 39 - 90%. Final fruit set of crosses were not measured successfully because; the late spring cold was destroyed by fruits of some crosses.

Highest fruit set mean was observed in the crosses of 03 × 105 with lowest fruit abscission. Crosses of 102 had highest fruit abscission, thus, initial fruit set percentage had no significant difference in the crosses (Table 3). Regarding the genotypes that are pollinated by different pollen type; final fruit set was not significantly affected by pollen type in all of the crosses (data not shown).

DISCUSSION

Results obtained from pollen tube growth pattern and fruit set in the crosses of 6 genotypes demonstrated that all of the genotypes were cross-compatible. Percentage of germinated pollen on the stigma was high when compared with the *in vitro* germination. This may be caused by the ideal condition on the stigma verses the *in vitro* conditions, including existence of proteins, amino acids and enzymes in the stigma or the lack of the calcium, boric acid and probably other ions in the *in vitro* medium.

Although, all the crosses showed significant differences in the pollen tubes number in the ovary, but, final fruit set was not affected by the pollen type. Means of the pollen tubes number in the ovaries were 3.2 - 9.1 in the crosses (Table 3).

High number of tubes in the ovaries and high fruit set, indicated the good compatibility of two genotypes 103 and 105, therefore, they could be introduced for orchard establishments. Many researchers studied the self- and cross-(in) compatibility of cultivars/genotypes using fruit set and fluorescence microscopy methods, and reported self-(in) compatible and cross-(in) compatible cultivars/genotypes in almond species with different effects on fruit taints taints (Alonso and Socias i Company 2005; L´opez et al., 2004 and 2006; Socias i Company and Felipe., 1987). For instance, Ortega et al. (2004) following field studies showed that, although 'Marcona' cultivar and 'S_{5133}' genotype had similar pollen tubes in the style, the fruit set of 'Marcona' was higher than 'S_{5133}'.

This phenomenon expresses the inference of other factors (for example, pollen tube number) in the fruit setting processes. Dicenta et al. (2002c) following self- and cross-pollination of 6 self-compatible almond cultivars

Table 3. Comparison of means for fruit set percentage and pollen tubes number in the ovary of crosses.

Crosses	Initial fruit set (%)	Pollen tube number in the ovary
100 × 101	54.4^{bc}	3.2^{d}
100 × 102	60.2^{ab}	4.8^{cd}
100 × 103	47.8^{dc}	8.3^{b}
100 × 104	58^{c}	3.8^{d}
100 × 105	56.4^{c}	6.7^{ab}
101 × 102	54.6^{c}	4.7^{c}
101 × 103	63^{b}	7.4^{ab}
101 × 104	57.4^{c}	5.3^{bc}
101 × 105	52.7^{c}	6.5^{bc}
102 × 103	40^{d}	4.1^{c}
102 × 104	49.7^{dc}	3.6^{d}
102 × 105	45.6	3.9^{d}
103 × 104	65^{a}	6.4^{ab}
103 × 105	67.52^{a}	9.1^{a}
104 × 105	60^{b}	5.2^{bc}

Means in each column with same letters were not significantly different at 5% level. Final fruit set was not showed, because spring cold damaged fruits of some crosses.

(Antoneta, Laurann, Marta, Guara, S2332, and S4017) found that, self- or cross-pollination of cultivars not showed significant differences in pollen germination, pollen tubes number penetrated to ovary, fruit set, fruit traits and nut traits. Furthermore, Ortega et al. (2006) demonstrated that, some of the selections among 26 self-compatible almond genotypes and two cultivars (Lauranne and Marta) had differences in some of the fruit traits following cross- or self-pollination.

Consequently, in this work the pollen tube number in the ovary, initial and final fruit set of cross-pollination groups showed that, all of the genotypes were cross-compatible and could pollinate each other regarding the overlapping time of blooming.

Conclusion

This research concluded that 6 studied almond genotypes were cross-compatible; therefore, all of them could be used in breeding programs or orchard establishment for pollination of each other based on the objectives. Best cross-compatibility was observed between genotypes 103 and 105.

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